Effects of Lecithin Addition in Oil or Water Phase on the Stability of Emulsions Made with Whey Proteins

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The effects of lecithin addition in oil or water phase on the stability of oil-in-water emulsions made with 0.1 wt% whey protein and 10 wt% n-tetradecane at neutral and acidic pH were studied by monitoring the gravitational creaming and phase separation. The effects of lecithin addition on the interfacial behavior of β-lactoglobulin were also studied to compare with the results of emulsion stability. At neutral pH, crude phosphatidylcholine (PC) from egg yolk or soybean increased the stability of the emulsion made with protein and lowered the interfacial tension of protein films more effectively than pure egg PC. A more remarkable effect on both the emulsion stability and the interfacial tension was found when crude PC was added in the oil phase rather than in the water phase. The purity of lecithins and the way to add them are suggested to be very important to make a stable emulsion with protein. On acidic pH (4.5 or 3.0), the increased creaming or phase separation in a whey protein-stabilized emulsion, but the lowered interfacial tension of β-lactoglobulin films, were found upon the addition of pure or crude PC in oil or water phase. These results suggest that in acidic pH, densely packed films may be formed on a planar oil–water interface, but not on adsorbed layers around oil droplets in an emulsion.

Key words: emulsion stability; interfacial tension; whey protein; β-lactoglobulin; lecithin

Whey protein isolates (WPI) are widely used as food ingredients in bakery, dairy, and meat production because of their good functional and nutritional properties.1,2 Among them, excellent surface and emulsifying properties are interesting and have been studied in detail.1–10 The stability of emulsions made with protein is known to be influenced by a number of factors such as the way to prepare, phase volume fraction, pH, temperature, ionic strength, and added surface-active emulsifiers.2,6,11 As the lack of uniformity in composition of commercial WPI, the effect of WPI components is also an important factor in the stability of emulsions made with whey proteins.2,4,5,10

Lecithin is an important ‘natural’ small-molecule emulsifier available for widespread use in food processing applications.12,13 Lecithin is composed of many different phospholipids, and the two most abundant phospholipid species are phosphatidylcholine (PC) and phosphatidylethanolamine (PE). The most important commercial source of lecithin for food production is soybean. Lecithins purified from egg yolk also have been used widely for parenteral emulsions for drug delivery and intravenous nutrition.12–15 Lecithin has been used both to stabilize fine emulsions as the sole stabilizer and to make stable emulsions with another emulsifier, proteins. The effects of lecithin on the stability of protein-stabilized emulsions are reported with positive or negative effects depending on the amount and the kind of added lecithin.16–19 The displacement of lecithin to milk proteins from oil–water interface has also been studied with the results not as effective as other (water-soluble) surfactants.16,18,19

In most emulsions with protein, the primary stabilization is provided by the protein-containing adsorbed layers at the oil–water interface. Thus, understanding of interfacial properties of protein film is essential to make a stable emulsion. Interfacial tension, interfacial rheology, and interfacial concentration have been studied to monitor the interfacial behavior of proteins at air–water and oil–water interfaces.16,20–24 A monolayer technique has also been often used for understanding the interaction of lecithin with proteins,25,26 with results suggesting that the amounts of adsorbed protein on the film at the oil–water interface are influenced by lecithin addition.

As the pH affects the conformational changes of globular proteins, the pH of the aqueous phase is also important for the stability of emulsions made with proteins. It is known that proteins tend to aggregate at the pH of their isoelectric point, and that the pH to make emulsions should be away from the isoelectric point for the prevention of coalescence and creaming in protein-stabilized emulsions.5,9

A variety of tests for the stability of oil-in-water emulsion have been studied with no single satisfactory method. These include turbidimetric technique,27 conductivity measurement,28,29 measurement of the amount of oil or cream separated during a certain period of time with or without centrifugation,6,7 or the average particle size or particle size distribution.6–10,30,31 Recently, Fligner et al. suggested that the extent of gravitational and centrifugal creaming, and not average particle size, is an adequate predictor of short-term emulsion stability with emulsions made with milk proteins.30

In this experiment, we studied the effects of lecithin addition in oil or water phase on the stability of emulsions made with whey proteins at neutral or acidic pH. β-Lactoglobulin, the most abundant whey protein, is mainly used for the protein source. Stability was evaluated by measuring the extent of gravitational phase separation. The interfacial behavior of β-lactoglobulin with or without lecithin in oil or water phase was also studied by measuring
the interfacial tension.

Materials and Methods

Materials. Bovine $\beta$-lactoglobulin (Lot130, lot no. 51H7210) was obtained from Sigma Chemicals (U.S.A.). Three kinds of lecithin were also obtained from Sigma Chemicals: pure egg PC (P2772, $\approx 99\%$ 1,2-sn-phosphatidylcholine [PC], dissolved in chloroform), crude egg PC (P9671, $\approx 60\%$ PC, crude soybean PC (P3644, $\approx 40\%$ PC). Pure egg PC was used after removal of chloroform by $N_2$ gas flushing. The phospholipid contents measured by the method of Raheja et al. were 93 and 97% in crude egg and soybean PC, respectively. These crude lecithins were used without adjusting the difference in phospholipid contents. The phospholipid composition of crude lecithin analyzed by HPLC was as follows: crude egg PC, 54%, PE 43%, phosphatidylinositol (PS) 1.3%, unknown 1.7%; crude soybean PC, PC 67%, PE 23%, PI 2.6%, unknown 7.4%. Lyso-type of PC and PE, and phosphatidylserine were not detected. A sample of whey protein isolate (WPI) was a gift from Taiyo Kagaku (Japan) (San-lacto I-1) with a protein content $>95\%$ (together with $<3\%$ ash and $<1\%$ fat). As an oil phase, $n$-tetradecane ($>99\%$) from Sigma Chemicals or commercial corn oil from Nissin Syosuka (Japan) were used without further purification. Buffer solutions were prepared with analytical grade reagents from Wako Pure Chemical Industries (Japan) and double-distilled water.

Emulsion preparation. A $\beta$-lactoglobulin solution was prepared with 0.02 M bis-tris buffer (pH 7.0) or 0.02 M acetate buffer (pH 4.5 or 3.0). A protein-stabilized oil-in-water emulsion was prepared at room temperature by homogenizing 0.3 g of $n$-tetradecane with 2.7 g of $\beta$-lactoglobulin solution at 15,000 rpm for 3 min in a Polytetramon homogenizer and then sonicating for 1 min with a ultrasonic generator (Nihonseiki Kaigo, Model US-50) at maximum power. The emulsions were degassed for 30 min using a water pump to remove entrapped air bubbles. The emulsions with 0.1 wt% $\beta$-lactoglobulin and 10.0 wt% oil, which were found to be unstable (Fig. 1), were used mostly in this experiment to study the stabilizing effect of added lecithin. In some cases, 0.1 wt% of lecithin was added before emulsification by dissolving or dispersing in the oil or water phase. WPI was also used as a protein source instead of $\beta$-lactoglobulin, and corn oil as an oil source instead of $n$-tetradecane.

Emulsion stability. Emulsion samples were transferred into a 7-mm i.d. and 100-mm length test tube, tightly capped and stored in a water bath at 25 ± 1°C. Creaming and/or coalescence behavior in samples was followed by monitoring visually the changes in the length of distinct cream and/or oil layers at the top and water layer at the bottom of the stored emulsions.

Interfacial tension. Interfacial tension at the $n$-tetradecane–water interface was measured using a Wilhelmy plate surface tensiometer (Kyowa Interface Science Co., CBV-P-A3) with a cell thermostatted at 25°C. $\beta$-lactoglobulin (0.01% w/v) dissolved in 0.002 M bis-tris buffer (pH 7.0) or 0.002 M acetate buffer (pH 4.5) was used as a water phase, and the oil phase was put on it. In some experiments, lecithin (10 -3 wt%) was added by dissolving in the oil or water phase before the start of the experiment.

Results and Discussion

The stability of oil-in-water emulsions made with $\beta$-lactoglobulin or WPI was strongly dependent on the concentration of the emulsifier protein (Fig. 1). $\beta$-Lactoglobulin-stabilized emulsion started to cream soon after the emulsification at the concentration of 0.1 or 0.2%. Emulsion with 0.5 or 1.0% $\beta$-lactoglobulin was stable without creaming for 6 h, and after that slightly creamed. In contrast, the WPI was found to make a stable emulsion at a concentration of 0.5% without creaming for 5 days. Among whey proteins, $\alpha$-lactalbumin is more surface active than $\beta$-lactoglobulin and bovine serum albumin.23 Other surface-active components of WPI are small molecule emulsifiers, phospholipids. The stabilizing effects of these surface-active components have been studied with uncertain results because of the lack of uniformity in composition of commercial WPI.2,4,5,10

As expected, emulsions with corn oil are more stable than emulsions with $n$-tetradecane. The facts that triglycerides are more surface active than pure carbohydrate, and that commercial corn oil contains surface active mono- and di-glycerides explain the stability of emulsion with corn oil. Although $n$-tetradecane is not used for food production, we used it as an oil source mostly in this experiment for experimental reproducibility.

The effects of lecithin addition on the stability of emulsions made with $\beta$-lactoglobulin at neutral and acidic pH are summarized in Table. At neutral pH, the addition of three types of lecithin in the water phase before emulsification is effective to improve the stability, but insufficient to prevent creaming. The effects of added lecithin are seen to be markedly dependent on the purity and the source of lecithin used. Emulsions made with $\beta$-lactoglobulin are more stable by the addition of crude lecithin than the pure lecithin, and by the addition of crude lecithin from soya beans than from egg yolk. The high contents of both minor components of phospholipids other than PC and surface-active impurities other than phospholipids may be the reason why crude soybean lecithin is a more effective stabilizer than the others. Rydhag and Wilton have reported that crude lecithin is more effective than pure lecithin as a sole emulsifier, and emulsions made with pure lecithin are very unstable.14 They suggested that the minor ionic surfactants like PA and PS in crude lecithin have a stabilizing action via their electrostatic and hydration forces. Fang and Dalgleish suggested the importance of the kind and purity of added lecithin by showing the stabilizing effects of egg yolk PC16 and destabilizing effects of synthetic lecithin, dipalmitoyl PC17 on the emulsion made with casein.

The stabilizing effects of crude PC at pH 7.0 were found to be more pronounced when added to the oil phase than when added to the water phase, and this is not the case with pure egg PC. So far, the method of lecithin addition to an emulsion has not been studied systematically; some results are with lecithin dissolved in the oil phase14,15 and the other is with lecithin dissolved in the water phase.17

![Fig. 1. Stability of Oil-in-Water Emulsions Prepared with $\beta$-Lactoglobulin or WPI.](image-url)
Table Effects of Lecithin Addition on the Stability of Emulsion Prepared with β-Lactoglobulin at Neutral or Acidic pH

<table>
<thead>
<tr>
<th>Lecithin</th>
<th>pH 7.0</th>
<th>pH 4.5</th>
<th>pH 3.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cream</td>
<td>Oil</td>
<td>Water</td>
</tr>
<tr>
<td>Not added</td>
<td>18.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(6.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lecithin was added in water phase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude Soybean PC</td>
<td>7.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Crude Egg PC</td>
<td>9.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pure Egg PC</td>
<td>14.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lecithin was added in oil phase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude Soybean PC</td>
<td>1.9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Crude Egg PC</td>
<td>3.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pure Egg PC</td>
<td>15.8</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Oil-in-water emulsion was prepared with 10 wt% n-tetradecane and 0.1 wt% β-lactoglobulin dissolved in 0.02 M bis-tris buffer (pH 7.0) or acetate buffer (pH 4.5 or 3.0). Commercial corn oil was also used as an oil phase (results in parenthesis). Lecithin was added in buffer solution or oil before emulsification at a concentration of 1.0 wt%. The stability of emulsions was measured by monitoring visually the changes in the length of each distinct layers, and expressed as volume percentages of cream or oil layer (top) and water layer (bottom) after incubation for 24 h.

For pure egg yolk lecithin, Fang and Dalgleish showed the similar mean droplet size of casein-stabilized emulsions with lecithin in the oil or water phase. Our results in the Table clearly show that the method to add a lecithin as an emulsifier is a very important factor, and crude PC should be dissolved in the oil phase to make a more stable emulsion with whey proteins.

Table also shows the effects of pH on the stability of emulsions made with β-lactoglobulin with or without lecithin. Emulsion made with β-lactoglobulin (pI = 5.2) alone at pH 4.5 were very unstable, and those made at pH 3.0 were not so unstable as at pH 4.5. Recently Hunt and Dalgleish also showed an increased instability of emulsions made with milk proteins at the pI values by the droplet size distribution technique. The solubility of proteins decreases and tends to aggregate at the isoelectric point. At near the isoelectric point (pH 4.5), β-lactoglobulin molecules on the surface of oil droplets may aggregate with each other not only on the same oil droplets but also on neighboring oil droplets, leading to creaming, flocculation and phase separation. Thus, the pH of the emulsion should be away from the isoelectric point to make a stable emulsion with protein.

At acidic pH (4.5 and 3.0), emulsions made with β-lactoglobulin were not stabilized by lecithins added in the oil or water phase. Separation of oil or water phase was found upon the addition of crude PC and an increased creaming was found upon the addition of pure PC. These results suggest that added lecithin in oil or water phase at acidic pH markedly weakens the adsorbed protein layers around oil droplets.

As the primary stabilization of emulsions made with protein is provided by the protein-containing adsorbed layers at the oil-water interface, the effects of lecithin addition in the oil or water phase on the interfacial tension of protein films at the planar oil-water interface were studied (Fig. 2). The tension at the interface of n-tetradecane and β-lactoglobulin solution at pH 7.0 was lowered by the addition of crude soybean PC, and the effect was stronger when lecithin was added in the oil phase than when added in the water phase. The effects of lecithin added in the oil phase as a sole surfactant are also strong.

The changes of tension at the interface of oil and β-lactoglobulin solution at pH 7.0 as a function of added crude soybean PC concentration are expressed in Fig. 3. The changes in the interfacial tension of lecithin alone dissolved in the oil or water phase are also shown. By the lecithin addition in oil phase even at a very low concentration, 10^-4%, the interfacial tension of β-lactoglobulin film was lowered markedly, suggesting the formation of densely packed layers with both lecithin from the oil phase and β-lactoglobulin from the water phase. However, the decrease in tension was rather moderate when crude soybean PC was dissolved in the water phase even at high concentrations. Then, lecithin dissolved in water phase seems to be not as effective to make interfacial layers with β-lactoglobulin. These results are consistent with the fact that the displacement of lecithin from water phase to milk proteins in the oil-water interface is not as effective as other (water-soluble) surfactants. In excess water, PC
exists as a mixture of lamellar mesophases and vesicles, and the less hydrophilic PE forms reversed hexagonal phases. This may be the reason for a lower surface activity of lecithin added in the water phase than that added in the oil phase with or without β-lactoglobulin.

The concentration of pure egg PC dissolved in water phase with and without β-lactoglobulin affected its interfacial tension very weakly (Fig. 4). Even when dissolved in the oil phase, a very high concentration is needed to lower the tension with or without β-lactoglobulin in the water phase. The less tendency of pure PC to lower interfacial tension than crude PC seems to be agreed well with the emulsion stability experiment.

Figure 5 shows that the interfacial tension of β-lactoglobulin at pH 4.5 continued to decrease for 24 h, and reached a lower value than pH 7.0 (Fig. 2). The β-lactoglobulin molecule near the isoelectric point will be more hydrophobic and compact in shape, and then arranged into interfacial film more slowly and densely than at pH 7.0. The considerable adsorption of β-lactoglobulin molecules on the surface of oil droplets at pH near the isoelectric point have already been reported.

The interfacial tension of β-lactoglobulin at pH 4.5 was lowered to a very low value by the lecithin addition in the oil or water phase (0.3 or 6.9 mN m⁻¹). Protein–lecithin complexes are known to be formed by sonication or lowering of pH. Although PC and PE are zwitterionic over a wide range of pH, acidic phospholipids (phosphatidic acid, PS, PI, etc.) are negatively charged, and then interact with positively charged β-lactoglobulin molecules at pH 4.5. This complexing of β-lactoglobulin with lecithin at the oil–water interface will make densely packed films and lower the surface tension. In the emulsion at pH 4.5, the increased complexing between β-lactoglobulin and lecithin in aqueous phase during sonication emulsification may weaken the film formation around oil droplets, and then destabilize the emulsion.

References
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